

SALINITY REACTIONS OF SOME FRESH- AND BRACKISH-WATER CRUSTACEANS

KARI LAGERSPETZ AND MAIJA MATTILA

*Department of Zoology and Lohm Marine Biological Station,
University of Turku, Turku, Finland*

Knowledge of the behavioral reactions of aquatic invertebrates to chemical stimuli has been reviewed by Warden, Jenkins and Warner (1940) and, more recently, by Hodgson (1955). The reaction thresholds of various invertebrate animals have been determined for various substances, especially for those known to stimulate the vertebrate chemoreceptors, *e.g.*, for several salts and alcohols. In addition, the part played by chemoreception in food recognition has been amply documented. The methods of study have been based either on the use of alternative chambers and gradient apparatus or on the conditioning of the behavior of the animals. In addition, the physiology of the chemoreceptors in some aquatic invertebrates has recently been studied by electrophysiological means (*Callinectes*, Hodgson, Lettwin and Roeder, 1955; *Limulus*, Barber, 1956; *Cambarus*, Hodgson, 1958).

The major part of the Baltic has a low and stable salinity. However, along the coasts of the northern Baltic, there occur periods of extreme dilution of the surface water, owing to the melting of snow and ice and the increased fresh-water output of the rivers in the spring. In such conditions, the capacity of selecting appropriate salinities could have a survival value for the brackish-water animals.

The behavioral discrimination of various salinities has been demonstrated in three semi-terrestrial crustaceans, *Ligia baudiniana* (Barnes, 1935, 1938, 1940), *Birgus latro* (Gross, 1955), and *Pachygrapsus crassipes* (Gross, 1957). In addition, Krijgsman and Krijgsman (1954) have found the South-African rock lobster, *Jasus lalandii*, to be capable of osmoreception. Earlier observations of Giersberg (1926) on *Octopus vulgaris* indicate that the reaction thresholds for salinity are in this species too high to allow the behavioral reactions to occur in natural conditions. Spiegel (1927) found that *Crangon vulgaris* reacted to higher concentrations of sea water than those found in its natural habitat.

As only a few studies dealing with the reactions of aquatic invertebrates to differences in the concentration of natural sea water were available, the salinity reactions of some fresh- and brackish-water crustaceans were studied by using an alternative chamber suitable for aquatic animals. The apparatus of Hodgson (1951) was adopted as the experimental device.

The following problems were studied: (1) Do the animals behaviorally discriminate between waters of different salinities? (2) Which are the concentrations preferred? (3) Are the reaction thresholds low enough to allow the reactions to play any part in the natural orientation of the animals? Additional information was sought on the following points: (4) Are the reactions to pure

NaCl solutions similar to those to diluted natural sea water? (5) Where are the receptors mediating the salinity reactions placed?

As test animals, the following crustaceans were used: *Asellus aquaticus* (Isopoda) both from fresh and brackish water, *Idotea baltica* (Isopoda) from brackish water, and *Gammarus* spp. (Amphipoda) from brackish water. It appeared that of the specimens of *Gammarus* used in experiments, 88% belonged to the species *G. oceanicus*, 7% to *G. locusta*, 3% to *G. zaddachi*, and 2% to *G. salinus*.

MATERIAL AND METHODS

For the experiments with fresh-water *Asellus*, the animals were caught in two shallow ponds in the parks of the city of Turku, southwestern Finland, in October, 1958, and in May and October, 1959. The brackish-water specimens of *Asellus* were collected in June, 1959, together with specimens of *Idotea* and *Gammarus* from the *Fucus vesiculosus* vegetation at Lohm Marine Biological Station in the Finnish southwestern archipelago, about 40 km. southwest from Turku. The experiments with brackish-water animals were performed at the Lohm Station. In the laboratory, the test animals were kept in their native, aerated water in polyethylene containers at room temperature (19–21° C.).

The apparatus of Hodgson (1951) was only slightly modified, i.e., the water flowing to the funnels D in Hodgson's Figure 1 came directly from two separate storage bottles containing the solutions in question. Care was taken to have the temperatures of the two solutions equal. In some preliminary experiments, water at different temperatures was allowed to flow through the sides of the reaction tube. When the difference between the temperatures was less than 5° C., the distribution of the animals in the apparatus did not deviate from the chance expectation. In the salinity reaction experiments, the temperature difference was always less than 0.1° C. The pH of the solutions was also controlled. Groups of 10 or 20 specimens were transferred to the alternative chamber through its left end, or, in every second experiment, through its right end. The number of animals on each side of the chamber was recorded at intervals of two minutes. At intervals of 10 minutes, the tubing screws of the apparatus were adjusted so that the solutions changed sides. The mean flow rate from the reaction tube to the sink was 200 ml. per minute. The duration of the experiment was 40 to 60 minutes. All experiments were performed at room temperature (19–21° C.). The control experiments with stained solutions showed that the solutions mixed only in a 0.5 cm. zone in the middle of the chamber. After the change of the sides, the distinct boundary was re-established in one minute. When one solution at the same temperature was allowed to flow through both halves of the chamber, the test animals showed no preference for either side. Except for specific purposes, the animals were used only once for the experiments. Altogether, 1200 test animals were used and their positions in the reaction tube were recorded 26,950 times.

RESULTS

1. Experiments with *Asellus* from fresh water

From the results of the experimental series 1, presented in Table I, it appears that the test animals were distributed at random in the chamber when the alternatives were tap water and Baltic brackish water with a salinity of 5.4‰.

The reaction threshold for pure NaCl was more closely studied, using fresh-water *Asellus*. The results of these experiments (series 2-6) appear in Table I. There are several ways of defining the threshold values, the most common being the intensity of the stimulus which evokes a response 50% of the times it is applied. Thus, if the animals in question have, through chemoreception and appropriate orientation mechanisms, selected one of the solutions in 50% of the cases when they have approached the boundary of the solutions, and the other 50% of the animals are distributed at random between the two solutions, 75% of the

TABLE I
Salinity reactions of Asellus aquaticus from fresh water

Exp. series	Test solutions	Number of test animals	Distribution of position records	Chi square	<i>p</i>
1	Tap water 5.4‰ B.W.*	30	311 289	0.8	0.4
2	Tap water 15‰ NaCl	60	944 256	394	<0.0005
3	Tap water 10‰ NaCl	60	908 292	316	<0.0005
4	Tap water 5‰ NaCl	60	814 386	153	<0.0005
5	Tap water 2‰ NaCl	30	346 256	14.1	<0.0005
6	Tap water 1‰ NaCl	30	477 273	55.5	<0.0005
7	5‰ NaCl 15‰ NaCl	60	831 369	178	<0.0005
8	5‰ NaCl 10‰ NaCl	60	679 503	31.2	<0.0005
9	10‰ NaCl 15‰ NaCl	60	700 500	33.3	<0.0005

* B.W., brackish water.

animals should be on one side and 25% on the other side of the boundary, when the threshold value for the reaction is reached. Another way of measuring the capacity for discriminative behavior is to determine the upper limit for the zone of indifferent reaction (U.L.I.R.). This is the maximal value of the stimulus which does not evoke a statistically significant response. The reaction threshold of fresh-water *Asellus* for NaCl was about 10‰, while the U.L.I.R. for tap water and NaCl was below 1‰.

The differential reaction threshold for pure NaCl was also studied with

TABLE II

Effect of antennectomy on the salinity reaction of Asellus aquaticus from fresh water

Exp. series	Test solutions	Number of test animals	Distribution of position records	Chi square	<i>p</i>
10	Tap water 15‰ NaCl	60 antennectomized	566 634	3.85	<0.05
11	Tap water 15‰ NaCl	60 antennectomized after 1-3 days	673 527	17.8	<0.0005

Asellus. The results of series 7-9 are given in Table I. The differential reaction threshold is defined as the difference of the stimulus values for which 50% of the animals show reaction, the others being distributed at random. The differential reaction threshold on both sides of 10‰ NaCl is more than 10‰ (i.e., higher than from 5 to 15‰). In these, as in the previous cases, specimens of fresh-water *Asellus* preferred the lower concentration alternative.

The position of the receptors responsible for the reaction to NaCl solutions was studied by recording the reactions of antennectomized specimens of *Asellus*. Series 10 was performed immediately after the removal of the antennae and antennulae and series 11 one to three days later with the same animals (Table II). When compared with the data from series 2, presented in Table I, it appears that antennectomy destroyed the reaction to NaCl. Thus, the chemoreceptors mediating this reaction are apparently situated for the most part on the antennae and/or antennulae in *Asellus*.

2. Experiments with *Asellus* from brackish water

The results of the experiments with brackish-water specimens of *Asellus* are presented in Table III. The results of series 12 show that even the specimens

TABLE III

Salinity reactions of Asellus aquaticus from brackish water

Exp. series	Test solutions	Number of test animals	Distribution of position records	Chi square	<i>p</i>
12	Tap water 6‰ B.W.	60	585 615	0.75	0.4
13	Tap water 10‰ NaCl	60	820 380	161.4	<0.0005
14	Tap water 5‰ NaCl	60	705 495	36.7	<0.0005
15	Tap water 1‰ NaCl	60	592 608	0.2	0.7

of *Asellus* living in the brackish water do not discriminate between tap water and their native 6‰ brackish water.

In order to determine the reaction threshold for pure NaCl solutions in brackish-water *Asellus*, experimental series 13–15 were performed. It was found that the reaction threshold is above 10‰, the U.L.I.R. for tap water and NaCl being about 1‰.

3. Experiments with *Idotea baltica*

For the sake of comparison, the reactions of a typical brackish-water isopod, *Idotea baltica*, were tested in tap water *versus* its native 6‰ brackish water. Sixty test animals were used. The animals were recorded 476 times on the side with fresh water and 724 times on the side with brackish water, the difference being significant (chi square 51.2, $p < 0.0005$).

4. Experiments with *Gammarus spp.*

As the bulk (88%) of the test animals belonged to the species *Gammarus oceanicus*, the results given here will probably mostly reflect the chemoreceptory conditions in this species. The results are presented in Table IV. *Gammarus* was the only animal used in this study which strongly reacted to the difference between tap water and 6‰ brackish water (series 17). As its preference for the brackish water seemed to be pronounced, its reactions to smaller salinity differences were tested for brackish waters with salinities of 5 *versus* 6‰ (series 18). The result, however, did not differ significantly from the chance expectation.

TABLE IV
Salinity reactions of Gammarus spp.

Exp. series	Test solutions	Number of test animals	Distribution of position records	Chi square	p
17	Tap water 6‰ B.W.	60	109 1091	804	<0.0005
18	5‰ B.W. 6‰ B.W.	60	577 623	1.76	0.2
19	Tap water 6‰ B.W.	60 without antennulae	320 880	261	<0.0005
20	Tap water 6‰ B.W.	60 without antennae	216 984	492	<0.0005
21	Tap water 6‰ B.W.	60 without antennae and antennulae	598 602	0.01	>0.9
22	Tap water 6‰ NaCl	60	546 654	9.7	<0.01
23	Tap water 6‰ NaCl	60 without antennae and antennulae	597 603	0.03	>0.9

In order to find the position of the chemoreceptors involved in the salinity perception in these amphipods, the antennulae were removed from 60 animals and the reactions of the animals to the difference between tap water and 6‰ brackish water were tested (series 19). When compared with the results of series 17, it was found that the intensity of the reaction was reduced. The intensity of the reaction can be calculated by the following formula:

$$\frac{A - B}{N} \cdot 100 = R.$$

If A and B represent the numbers of records of animals on each side of the reaction tube and N is the total number of position records, then R is the excess percentage of records on the side with A animals. The intensities of reaction measured as the excess percentages on the side with 6‰ brackish water, calculated for the experimental series 17 and 19-21, were as follows:

17: 6‰ brackish water vs. tap water, normal animals	+81.8%
19: as above, antennulae removed	+46.7%
20: as above, antennae removed	+64.0%
21: as above, antennulae and antennae removed	+ 0.3%
Chance expectation	0.0%

Thus, the removal of both antennae and antennulae totally abolished the salinity reactions in *Gammarus*. The removal of antennulae, only, reduced the intensity of the salinity reaction, as did the removal of the antennae, although to a lesser extent. It seems safe to assume that *Gammarus* has the chemoreceptors responsible for orientation in a salinity gradient on its pairs of antennulae and antennae, the antennulae being apparently somewhat more important in salinity perception.

It seemed worthwhile to explore whether *Gammarus*, which preferred brackish water with a salinity of 6‰ (expressed as NaCl), would react similarly to pure 6‰ NaCl solutions. Thus, the experiments of series 22 (Table IV) were performed. The intensity of the reaction was much weaker than for 6‰ brackish water, but still positive for the saline medium (excess percentage + 9.0%). The removal of antennulae and antennae (series 23) also in this case abolished the salinity preference reaction, the excess percentage being reduced to 0.5%. The receptors involved in the chemoreception of pure NaCl are thus also situated on the antennulae and/or antennae.

DISCUSSION

1. The significance of salinity reactions in nature

It was shown that the specimens of *Asellus* from fresh and from brackish water did not behaviorally react to the difference between tap water and Baltic brackish water with a salinity of 5.4‰ or 6‰. These salinities are approximately those which usually prevail in the natural habitats of *Asellus aquaticus* in wide areas of the northern Baltic. As no reaction occurs for this large difference, the com-

pensation for the much smaller salinity variations (occurring, for example, on the shores as a consequence of the melting of the ice in spring) by movement of the animals to other habitats seems utterly improbable.

Specimens of *Gammarus* clearly reacted to the difference between fresh water and 6‰ brackish water, but they did not show a significant response to the difference between 5‰ and 6‰ brackish water. The reaction of *Idotea baltica* to the difference between fresh water and 6‰ brackish water was even less pronounced than that of *Gammarus*. Thus, the adaptive value of behavioral reactions to differences in salinity in the crustaceans studied does not seem to have been established by the results of this study. However, the methods of investigation may be criticized for not allowing longer times than 10 minutes for the discriminative reactions to the alternatives studied. However, in several cases with pure NaCl solutions, this period of time was found to be entirely sufficient for the performance of definite responses by most of the animals. The possible significance of behavioral salinity reaction mechanisms in other truly aquatic brackish-water animals, for example in estuarine forms, deserves further attention.

2. The salinity reaction thresholds

Most of the experimental series were devoted to the determination of salinity reaction thresholds. It appeared that the reaction threshold for NaCl is somewhat higher for the brackish-water specimens of *Asellus* than for the fresh-water ones. Similarly, the U.L.I.R. between fresh water and NaCl is higher in the brackish-water specimens. It would be interesting to find out whether this is a result of a sensory or some other physiological adaptation process, or an indication of physiological race formation. As reported previously (Lagerspetz, 1958), and afterwards confirmed by repeated tests, the brackish-water specimens of *Asellus* do not survive a longer stay in fresh water, while the fresh-water specimens are apparently not injured by a transfer to Baltic brackish water. Thus, one could at least study the effects of the stay of the fresh-water specimens in brackish water on their salinity reaction threshold.

The experiments for the determination of the differential salinity reaction threshold in *Asellus* showed that these animals are not only capable of discriminating fresh water from saline solutions, but also react differently to different concentrations of NaCl.

Fresh water or the weaker NaCl solution was preferred by *Asellus*, which showed no significant reaction to the brackish water. However, the brackish-water specimens were rather more often found on the side with brackish water, and the fresh-water animals slightly more often on the side with fresh water. In all cases, the number of animals on the brackish-water side was significantly higher for brackish-water animals. *Gammarus* slightly but significantly preferred 6‰ NaCl to fresh water. Such a comparatively strong NaCl solution was very definitely rejected by fresh-water *Asellus*, but also to a lesser degree by the brackish-water specimens. The reaction of *Gammarus* in brackish-water *versus* fresh-water experiments was again strongly positive towards brackish water. Thus, the following modifications may be linked with the progressive adaptation of fresh-water animals to brackish water:

- (1) The rise of the NaCl reaction threshold by the development of a slight preference for dilute solutions of NaCl to fresh water.
- (2) The development of a strong preference for brackish water to fresh water.

3. *The location of chemoreceptors*

The location of chemoreceptors in Crustacea has been documented and discussed in some twenty papers since 1887. Most experiments hitherto made have been performed on Decapoda. Among the exceptions are the early observations of May (1887) on *Mysis flexuosa* (Mysidacea), studies by Abraham and Wolsky (1930) on land isopods, by Uchida (1930) on the sex recognition in *Asellus*, and by Seifert (1930) on *Triops cancriformis* (Branchiopoda).

The chemoreceptors may be specialized to mediate responses only for certain chemical substances. Thus, the different receptor types may also have different sites in the animal. The results of experiments with one or a few substances cannot thus be generally applied to the receptors for other substances. It is therefore understandable that different authors have attained different results, even when working with the same species. In decapods, antennulae, antennae, mouth parts, tips of the appendages and the entire body surface have been variously described as the sites of chemoreceptors. Most of the evidence supports the special importance of the external ramus of the antennulae for the "distance" chemoreception, and of the mouth parts and other appendages for the contact chemoreception. The role of the receptors on the antennulae of *Callinectes* and *Cambarus* in chemoreception has also been proved through the electrophysiological studies of Hodgson, Lettwin and Roeder (1955) and Hodgson (1958).

As the removal of the antennae and antennulae in *Asellus* at first apparently abolished the reaction to NaCl, the chemoreceptors mediating the response seem generally to be situated on the antennae and/or antennulae. However, the results obtained for experiments performed one to three days after the amputation of the antennae and antennulae show that some sensitivity to NaCl may still exist in the animals or is rapidly regained.

More clear-cut results were obtained in the experiments with *Gammarus*. In these, the salinity receptors involved in reactions to brackish water are apparently on the antennulae and antennae. The removal of these also abolished the reaction to pure NaCl solutions. Thus, in amphipods the antennulae and antennae, and in isopods the antennulae and/or antennae, seem to contain the salinity receptors, and the results thus corroborate the various earlier observations on the location of the "distance" chemoreceptors in Crustacea.

It is tempting to think that "on terrestrial organisms the humidity has probably similar physiological effects as the salinity on aquatic organisms" (Kinne, 1957, p. 90). However, the problem faced by aquatic animals seems to be more in the maintenance of the proper ionic composition of their body fluids, while in terrestrial animals it is in the maintenance of a proper degree of dilution of the body fluids, already controlled for their ionic equilibrium. A more specific argument emerges from the present study: in *Asellus* the site of the salinity receptors is on the antennae and/or antennulae, which have recently been shown to be insignificant for the orientation of *Asellus* in an alternative chamber with different humidities of the air (Lagerspetz and Lehtonen, 1961).

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SUMMARY

1. The salinity reactions of *Asellus aquaticus* from fresh and Baltic brackish water (6‰), of *Idotea baltica* and of *Gammarus* spp. from brackish water were studied with the apparatus of Hodgson (1951). Both natural brackish-water and pure NaCl solutions were used.

2. None of the experimental animals had, for brackish water, reaction thresholds low enough to allow behavioral selection of salinities to occur in natural conditions.

3. The reaction threshold for NaCl solutions was higher in brackish-water than in fresh-water specimens of *Asellus*. *Asellus* always preferred the more dilute concentration. *Idotea baltica* and *Gammarus* preferred brackish water to fresh water. *Gammarus* preferred even 6‰ NaCl to fresh water.

4. In *Asellus*, the chemoreceptors mediating the response to salinity variations seem, for the most part, to be situated on the antennae and/or antennulae. In *Gammarus*, the salinity receptors are situated on the antennae and antennulae.

LITERATURE CITED

- ABRAHAM, A., AND A. WOLSKY, 1930. Die Geruchsorgane der Landisopoden. *Zeitschr. Morphol. Ökol.*, **17**: 441-463.
- BARBER, S. B., 1956. Chemoreception and proprioception in *Limulus*. *J. Exp. Zool.*, **131**: 51-73.
- BARNES, T. C., 1935. Salt requirements and orientation of *Ligia* in Bermuda. III. *Biol. Bull.*, **66**: 259-268.
- BARNES, T. C., 1938. Experiments on *Ligia* in Bermuda. V. Further effects of salts of heavy sea water. *Biol. Bull.*, **74**: 108-116.
- BARNES, T. C., 1940. Experiments on *Ligia* in Bermuda. VII. Further effects of sodium and magnesium. *Biol. Bull.*, **78**: 35-41.
- GIERSEBERG, H., 1926. Über den chemischen Sinn von *Octopus vulgaris* Fabr. *Zeitschr. vergl. Physiol.*, **3**: 827-838.
- GROSS, W. J., 1955. Aspects of osmotic regulation in crabs showing the terrestrial habit. *Amer. Nat.*, **89**: 205-222.
- GROSS, W. J., 1957. A behavioral mechanism for osmotic regulation in a semi-terrestrial crab. *Biol. Bull.*, **113**: 268-274.
- HODGSON, E. S., 1951. Reaction thresholds of an aquatic beetle, *Laccophilus maculosus* Germ., to salts and alcohols. *Physiol. Zool.*, **24**: 131-140.
- HODGSON, E. S., 1955. Problems in invertebrate chemoreception. *Quart. Rev. Biol.*, **30**: 331-347.
- HODGSON, E. S., 1958. Electrophysiological studies of arthropod chemoreception. III. Chemoreceptors of terrestrial and fresh-water arthropods. *Biol. Bull.*, **115**: 114-125.
- HODGSON, E. S., J. Y. LETTWIN AND K. D. ROEDER, 1955. The physiology of a primary chemoreceptor unit. *Science*, **122**: 417-418.
- KINNE, O., 1957. A programmatic study of comparative biology of marine and brackish-water animals. *Année Biol.*, **33**: 87-92.
- KRIJGSMAN, B. J., AND N. KRIJGSMAN, 1954. Osmorezeption in *Jasus lalandii*. *Zeitschr. vergl. Physiol.*, **37**: 78-81.
- LAGERSPETZ, K., 1958. The brackish-water tolerance of some freshwater crustaceans. *Verh. internat. Ver. Limnol.*, **13**: 718-721.
- LAGERSPETZ, K., AND AILA LEHTONEN, 1961. Humidity reactions of some aquatic isopods in the air. *Biol. Bull.*, **120**: 38-43.

- MAY, K., 1887. Über das Geruchsvermögen der Krebse nebst einer Hypothese über die analytische Tätigkeit der Riechlärchen. Diss. Kiel.
- SEIFERT, R., 1930. Sinnesphysiologische Untersuchungen am Keimfuß (*Triops cancriformis* Bosc.). *Zeitschr. vergl. Physiol.*, **11**: 386-436.
- SPIEGEL, A., 1927. Über die Chemorezeption von *Crangon vulgaris* Fabr. *Zeitschr. vergl. Physiol.*, **6**: 688-730.
- UCHIDA, T., 1930. Observations sur le sens de la reconnaissance du sexe chez *Asellus*. *Bull. Soc. Zool. France*, **55**: 516-520.
- WARDEN, C. J., T. N. JENKINS AND L. H. WARNER, 1940. Comparative Psychology. Vol. II. Plants and Invertebrates. The Ronald Press, New York.